FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE
HOST-PATHOGEN INTERACTIONS

Impact of Inhibition of the TCA cycle on ATP and NADH levels in *Escherichia coli* during Bacterial Replication

Raina De La Cruz, Briana Mercado, Aiswarya Suriaprakash, and Jessica O'Hara

EXPANSION OF RESEARCH

- Quantify ATP, NADH, and glucose levels before and after cell lysis to further understand the impacts of each knockout on cellular metabolism and the resulting impact on T4 replication.
- Expand range of genes in the TCA cycle to manipulate (*gltA, maeA, acnB, sucC*)
  - This will be quantified by utilizing a plate reader (@600nm) in both growth and lysis curves over eight hours.
  - The growth curve will measure the growth of the new genes compared to wildtype (WT) *E. coli*
  - The double agar overlay method will be utilized to grow and analyze T4 and T4r bacteriophage growth with the wild type and gene knockout of choice.

Gene	TCA Cycle Enzyme
gltA	Citrate Synthase
maeA	Pyruvate Dehydrogenase
acnB	Aconitase
sucC	Succinyl-CoA Synthetase

Objective: Understand ways to treat bacterial diseases in the face of antibiotic resistance, specifically phage therapy.

INTRODUCTION TO *FUMA* AND *ICD* GENES

The tricarboxylic acid (TCA) is the metabolic pathway that both genes of interest are part of. The TCA cycle consists of a series of reactions that occur within aerobic respiration. This is not the most ATP generative part of aerobic respiration; rather, it creates high energy carriers, NADH and $FADH_2$.

The *fumA* gene is one of the three fumarase genes that creates the enzyme need for the conversion of fumarate to S-malate, in the last few processes of the TCA cycle.

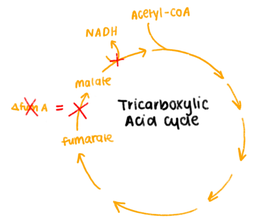


Figure 1. *ΔfumA* in the TCA cycle Illustration of the TCA cycle and gene knockout implications.

icd
Isocitrate ⇒ α-ketoglutarate ⇒ Normal ATP
Isocitrate ⇏ ~~α-ketoglutarate~~ ⇒ Decreased ATP
~~*icd*~~

Figure 2. Δ*icd* Reaction in Krebs Cycle. Diagram of the TCA cycle and gene knockout implications.

The *icd* gene connects the TCA cycle to the glyoxylate bypass pathway. It is the first enzyme in a bacterial pathway to be regulated by phosphorylation.

PLAQUE ASSAYS SHOW BACTERIAL LYSIS

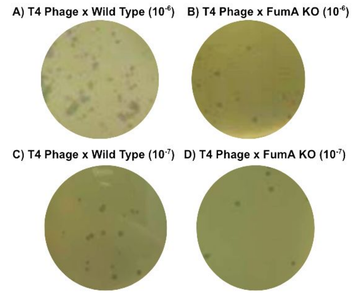


Figure 3. *ΔfumA* plaque assays
A double agar overlay method was used with T4 bacteriophage in the wild type (A and C) and *ΔfumA E.coli* strains (B and D) at dilutions of 10^{-6} (A and B) and 10^{-7} (C and D). There are less PFU in the *ΔfumA* strain than the wild type.

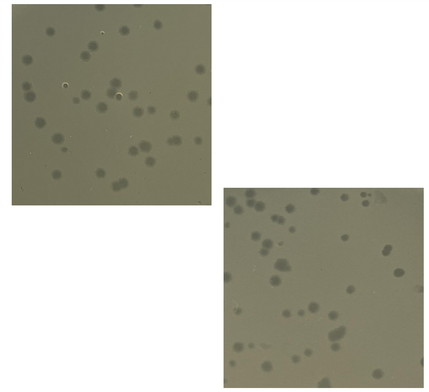
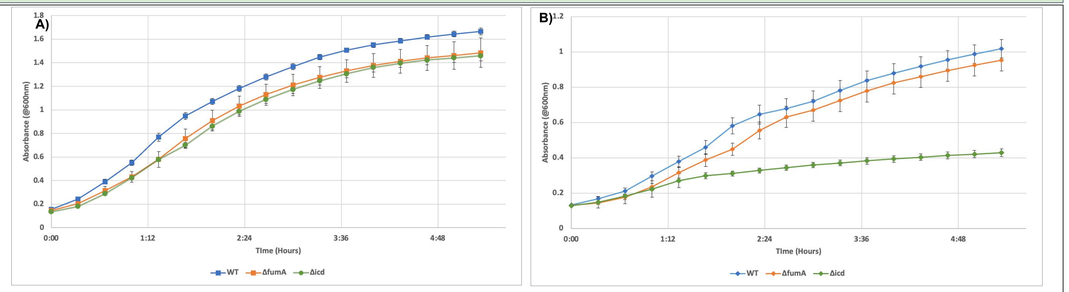


Figure 4. *Δicd* plaque assays
The plaque assays feature plaques of similar size and shape, indicating similar lysis of bacterial cells by phage.

ΔFUMA AND ΔICD LED TO DELAYED BACTERIOPHAGE REPLICATION

Figure 5. Growth Curves
A) Growth of Δicd and ΔfumA in LB Media
Growth of both knockout strains were not impacted in LB media.
B) Growth of Δicd and ΔfumA in M9 Media
Growth of the *Δicd* strain was significantly less in M9 media.



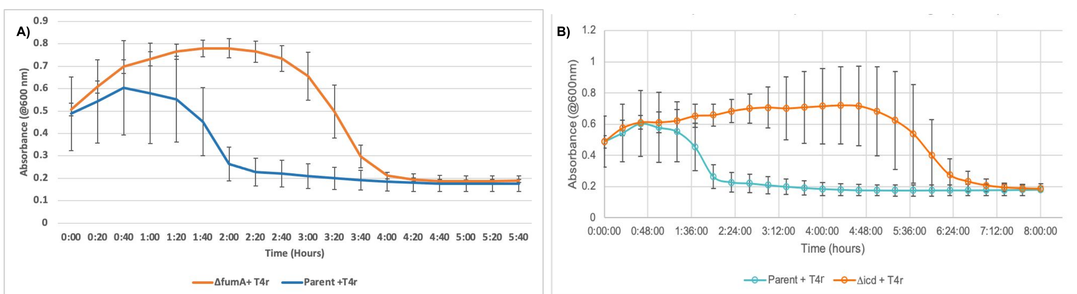


Figure 6. Lysis Curves
A) ΔfumA
Lysis of the parent and *ΔfumA* strain with T4 phage was observed over time by measuring absorbance at 600nm. The *ΔfumA strain* experienced slower lysis by phage than the parent strain.
B) Δicd
Lysis of the parent and *Δicd* strain with T4r phage was observed over time by measuring absorbance at 600nm. The *Δics strain* experienced slower lysis by phage than the parent strain

ΔICD FOUND TO DECREASE ATP LEVELS IN *E. COLI*

- Deletion of the *icd* gene led to reduced specific growth rate and reduced specific glucose consumption rate because of the lower intracellular ATP/ADP and NADPH/NADP+ ratios compared to the parent strain[5].
- Since *ΔfumA* followed the same trends in growth and lysis curves, it is assumed that the quantification of ATP/ADP and NADPH/NADP+ ratios in this strain and other TCA cycle enzyme knockout strains will provide similar results.

QUANTIFICATION METHODS

These assays will be performed to quantify NAD-NADH ratios, glucose consumption rate, and ATP levels during growth and viral replication.

NAD-NADH Pool Isolation and NAD Cycling Assay[6]	Sigma-Aldrich Glucose (HK) Assay Kit[4]	ATP Assay Kit (Colorimetric)[1]
1. Samples will be centrifuged and the subsequent supernatant will be removed and will immediately be frozen in a dry ice-ethanol bath. 2. HCl (for NAD extraction) or NaOH (for NADH extraction) is added to the pellets. 3. Cycling assays will be performed for both, the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-di phenyl tetrazolium bromide (MTT) is directly proportional to levels of NAD and NADH @ 570 nm.	1. Glucose is phosphorylated by ATP with the help of hexokinase → Glucose-6-Phosphate (G6P) and ADP are products. 2. G6P is oxidized to 6-phosphogluconate in the presence of NAD in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH) 3. During the oxidation, an equimolar amount of NAD is reduced to NADH. 4. The resulting increase in absorbance @ 340 nm is directly proportional to the concentration of glucose. 5. Glucose consumption rate is measured by measuring glucose concentration (intra or extracellular) over time.	1. ATP is made through metabolic reactions in the cells → ATP is then used to phosphorylate glycerol. 2. The phosphorylation of glycerol reaction produces byproduct (Resorufin) → byproduct is then stained red via assay and able to be quantified by optical density (OD) readings @ 570 nm.

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